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Phytophthora Species in Malaya.

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The following account is a record of further progress in the study of local species of *Phytophthora* concerning which a preliminary report was published in February, 1928. (1) With the exception of two species, all the local species in culture have been isolated from *Hevea brasiliensis* (H.B.K.) Muell—Arg., from the diseases known as Black Stripe, Patch Canker, and Pod-rot. The results prove that several species of *Phytophthora* are directly responsible for these diseases in Malaya, while experiments with certain other species from different hosts indicate that they also may be causal organisms. The other two species of *Phytophthora* were isolated from a disease of Roselle fibre (*Hibiscus sabdariffa L. var. altissima*), and from a disease of Sireh—the betel vine (*Piper Betle L.*). Diseases caused by *Phytophthora* are of importance in Agriculture all over the world, and, since our knowledge of the local species is scanty, it is desirable to gather together some facts concerning this genus in Malaya.

In addition to the crops mentioned above, *Phytophthora* species are known to attack coconut palms, and areca palms, castor oil, tobacco, tomato, and potato plants, nutmeg, cinnamon and citrus trees, and other tropical crops. A knowledge of the local species is therefore of the greatest importance, in case of any of these crops become attacked by a *Phytophthora* species.

The fungus *Phytophthora* reproduces by means of sexual and non-sexual spores. The sexual spores, called oospores, are not always formed by the different species, but, when formed, they serve to carry the fungus over a period of unfavourable conditions such as drought. The non-sexual spores are of two kinds—sporangia and chalmydospores—and are normally aerial. The sporangia require the presence of moisture in order to form motile bodies called zoospores, which are considered to be the means by which the fungus spreads rapidly. Zoospores are formed inside the sporangia and escape singly or in groups through the papillae. (Plate 3). They possess cilia by means of which they swim about in the water. After a short active period they come to rest and germinate, and, if they are near a suitable host plant, the germ tube penetrates through the epidermis and the the plant may become diseased. Sometimes the sporangia germinate directly without forming zoospores, in which case the germ tubes from the sporangia may start the infection. Chlamydospores are thicker walled than sporangia and do not germinate readily. They are not formed by every species, and, like oospores, act as resting bodies which can withstand unfavourable conditions. They do not form zoospores, but germinate directly.

Since the spread of the fungus *Phytophthora* is largely controlled by the presence and absence of moisture, epidemics of diseases causes by the fungus do not appear in dry weather.

In the following pages, reference is frequently made to *Hevea*. Since only one species of *Hevea* is concerned—*H. Brasiliensis* Muell.—the name *Hevea* refers to this species.

History of the local and other species of Phytophthora.

The disease known as Black Stripe of *Hevea* was first recorded in Malaya in 1916 (2). This was the first record of a *Phytophthora* sp. causing a disease of an economic crop in this country.

In 1918 R. M. Richards isolated a fungus, which he considered to be a *Phytophthora* sp. from Patch Canker of *Hevea*. A description of this fungus was not published, but, in a report to the Brown Bast Investigation Committee, of which he was a member, Mr. Richards stated that "from the characters of the antheridium, and its relationship to the oogonium, it is clear that my fungus must be placed in the 'cactorum' group." Also, in a letter to the Secretary of the

above Committee he mentions a series of inoculations in which he had to use "oospore material" as all his cultures were in that condition. This is the only record of a local species with paragynous antheridia, and it is possible that Richards was dealing with a *Pythium* sp. isolated from Patch Canker of *Hevea* by the writer in 1924 (3).

In 1925 the writer obtained a *Phytophthora* from Sireh (*Piper Betle* L.) A note on this fungus was published in 1926 (4) and again in 1928 (1). This species has been identified as *Phytophthora colocasiae* Rac.

In the same year two different species of *Phytophthora* were isolated from two bark diseases of *Hevea* i.e. Black Stripe and Patch Canker. The species from Black Stripe was obtained from Malacca and was identified as *P. palmivora* Butl.,* and the species from Patch Canker was obtained from Selangor. These two fungi are entirely different in their growth in culture. About the same time *P. palmivora* Butl. was isolated from Patch Canker of *Hevea* in North Perak. It was indistinguishable from the strain from Black Stripe of *Hevea* in Malacca. This species was again isolated from Black Stripe of *Hevea* in Chemor, Perak, in 1928.

In 1927 the writer obtained a *Phytophthora* from some diseased *Hevea* pods in Selangor. This fungus produces oospores with amphigynous antheridia, in large numbers in culture, as well as sporangia. It is a new species.

In 1928 this fungus was again isolated from Black Stripe of *Hevea* in Malacca. In this year also another *Phytophthora* was obtained from Black Stripe of *Hevea* in Negri Sembilan, and yet another was isolated from a disease of Roselle fibre (*Hibiscus sabdariffa var altissima* L.)

This concludes the list of the local species which have been obtained up to the present.

In addition to the local species a number of other *Pytophthorae* have been obtained and grown in culture, in this

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* Formerly known as *P. faberi* Maubl.

laboratory, to assist in comparing the different species. For convenience, all the species have been numbered as follows:—

No. 1	*P. palmivora* Butl. Malacca S.S. Black Stripe, *Hevea*.	1925.
No. 2	*P. palmivora* Butl. Chemor, Perak, F.M.S. Black Stripe, *Hevea*	1928.
No. 3	*Phytophthora* sp. Negri Sembilan F.M.S. Black Stripe, *Hevea*.	1928.
No. 4	*Phytophthora* sp. Malacca, S.S. Black Stripe, *Hevea*.	1928.
No. 5	*Phytophthora* sp. Selangor F.M.S Pod-rot, *Hevea*.	1927.
No. 6	*Phytophthora* sp. Selangor F.M.S. Patch Canker, *Hevea*.	1926.
No. 7	*Phytophthora meadii* McRae, India, Pod-rot and leaf fall of *Hevea*.	1928.
No. 8	*Phytophthora* sp. Selangor F.M.S. Wilt, Roselle fibre (*Hibiscus sabdariffa* L.)	1928.
No. 9	*P. colocasiae* Rac. Pahang F.M.S. Wilt of Sireh (*Piper Betle* L.)	1925.
No. 10	*P. colocasiae* Rac. U.S.A. via Kew	1926.
No. 11	*P. palmivora* Butl. India, Bud-rot, Coconut Palm.	1924.
No. 12	*P. arecae* Coleman, India, Areca Palm.	1928.
No. 13	*P. parasitica* Dastur, India, Castor Oil.	1928.
No. 14	*Phytophthora* sp. India, *Piper Betle* L.	1928.
No. 15	*P. nicotianae* B. de H. Florida, Tobacco, via Kew.	1926.
No. 16	*P. nicotianae* B. de H. Sumatra, Tobacco.	1928.
No. 17	*P. nicotianae* B. de H. Java, Tobacco, via Kew.	1926.
No. 18	*P. palmivora* Butl. Jamaica, Coconut Palm, via Kew.	1926.
No. 19	*P. palmivora* Butl. St. Vincent, Cotton, via Kew.	1926.
No. 20	*P. palmivora* Butl. Trinidad, Cacao, via Kew.	1926.
No. 21	*P. cinnamomi* Rands, Java, Cinnamon, via Kew.	1926.

When reference is made to species not included in this list, they are marked by the letters (N.L.) i.e. not listed.

In the above list *P. palmivora* Butl. refers to the type species No. 11, which was received from India, and to those strains which have been known in the past as *P. faberi* Maubl. since it is now considered that the two are the same species. On ground of priority the name should be *P. palmivora* Butl.

No. 1 Phytophthora palmivora Butl. obtained from Black Stripe of Hevea Brasiliensis in Malacca, 1925.

This fungus was isolated by cutting away diseased bark until diseased wood was reached. Pieces of this wood were placed in test tubes, containing Maize Agar, and in some of them a pure growth was obtained in a few days. The fungus was subcultured on to various media and kept in culture for several months before it was studied in detail.

Growth on Quaker Oats Agar.

In three days a quantity of lanate aerial mycelium is produced. The mycelium is non-septate and hyaline; sporangia and chlamydospores are present in considerable numbers.

Maize Agar.

Aerial mycelium is produced in quantity after three days; it tends to be slightly shorter than on Quaker Oats Agar. Sporangia and chlamydospores are present in large numbers, the latter predominating.

Green Pea Agar.

Very little aerial mycelium is produced in three days, but the surface growth is good. Sporangia are exceedingly numerous on the surface of the medium. Chlamydospores are present in less numbers. After fourteen days there is more development of aerial mycelium, mainly confined to the lower portion of the slant.

Potato Dextrose Agar.

On this medium there is a strong growth of aerial mycelium; sporangia and chlamydospores are present in large numbers. The mycelium presents a smoother appearance than on the other media. If grown for a long time on this medium, sporangia production diminishes.

Potato Agar.

Aerial growth is good in four days, but the mycelium is short. Chlamydospores are produced in considerable numbers. The sporangia are less numerous and some of them tend to be almost spherical, differing from chlamydospores by the presence of papillae, and in having slightly thinner walls.

Potato Blocks in Roux Tubes.

After four days there is a moderate amount of lanate aerial mycelium. A large number of sporangia are produced, some long and narrow, others almost spherical, but the majority conform to type. Chlamydospores are also present in quantity. The potato cylinder becomes discolored greyish black in three weeks. (Bacteria were suspected but none were present).

Baked Slices of Young Papaya Fruit.

There is a slight growth of aerial mycelium, and large numbers of sporangia and chlamydospores are produced on the surface of the slices.

No oospores were formed on any of these media.

DESCRIPTION.

The sporangia are, in the majority of cases, pearshaped with prominent rounded papillae. They are inclined to be narrowly ovate—mean ratio of length to width being 1.66 or over,—but a few almost spherical forms can be found especially on potato media. The conidiophores are usually inserted at the base and a short portion frequently remains attached to the sporangia when they are shaken loose from the parent hyphae. A few obpyriform types with much reduced, or no papillae may be formed.

Chlamydospores are thick walled and circular and may have a large central vacuole. They may develop in clusters of three or four, on short pedicels, from circular swellings in the hyphae.

Measurements of the sporangia and chlamydospores were obtained from cultures which produced them freely and early —usually after the fifth day. The media used were Potato-dextrose, Maize, Green Pea, and Quaker Oats Agars and Potato blocks in Roux tubes. The means obtained from Quaker Oats Agar were somewhat larger than those from the other media. The figures are given in Table 1.

TABLE 1.

Details of Sporangia and Chlamydospores of No. 1 (P. palmivora Butl. from Black Stripe of Hevea (Malacca).

SPORANGIA.

Medium	Number measured.	Range in Microns.	Mean in Microns.	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
		L x W			
Potato-Dextrose Agar	200	30-75 x 18-42	49.70 x 30.27	1.15-2.33	1.66
Maize Agar ...	100	36-78 x 23-39	50.63 x 29.49	1.25-2.33	1.71
Green Pea Agar	100	33-66 x 27-39	48.81 x 31.49	1.16-2.12	1.55
Quaker Oats Agar ...	100	39-75 x 24-45	56.61 x 33.09	1.23-2.50	1.72
Potato Block ..	100	36-72 x 24-39	50.25 x 30.27	1.06-2.22	1.67

CHLAMYDOSPORES.

Medium.	Number measured.	Range in Microns.	Mean in Microns.
Potato-Dextrose Agar...	100	21-45	33.06
Maize Agar ...	100	27-48	36.01
Green Pea Agar ...	100	27-48	36.75
Quaker Oats Agar ...	100	27-48	37.58
Potato Block ...	100	30-45	36.45

Inoculation Experiments.

No. 1—Six rubber seedlings, two months old, were inoculated at the collar, using mycelium and zoospores. Three seedlings were wounded and three were unwounded. The inoculations were kept moist with cotton wool for fourteen days. After three months no external signs of disease were noticed.

No. 2—Six of the same batch of seedlings were inoculated, in the same way, when they were seven months old. No external signs of disease were noticed after one month, but on splitting open the stems of the wounded seedlings a discoloration of the wood was noticed, extending about one inch up from the wound. The fungus was recovered in culture from the discolored wood.

No. 3—Leaves of two rubber seedlings were inoculated by placing a portion of Agar medium, on which the fungus was growing, on to the leaves. Some of the leaves were wounded. The plants were covered by a bell-jar to keep the atmosphere moist. The result was negative.

No. 4—The experiment was repeated, using zoospores, and the leaves were folded to hold water. After ten days there was no result.

No. 5—Three young *Hevea* pods were wounded and inoculated with the fungus. In four days there was a strong growth of mycelium on the outside of the pods and in ten days the pods were badly rotted. The fungus was recovered in culture.

No. 6—Three *Hevea* pods, unwounded, were inoculated by placing a suspension of mycelium and zoospores in the hollow formed at the stalk end. After a week the mycelium of the fungus began to appear all over the pods. Soon after, contaminations (*Gloeosporium* sp. etc.) were present, and the pods rotted away. The *Phytophthora* was recovered in culture along with numerous contaminations.

No. 7—The tapping cuts of three *Hevea* trees were inoculated by smearing a portion of Agar medium, on which

the fungus was growing, over the recently tapped bark. The inoculations were covered with moist cotton wool. A control was made by smearing Agar medium only over the tapped bark of three other trees and covering similarly. The trees were tapped daily, and, after three days, the bark of the inoculated trees was discolored with greenish black lines which had extended into the wood. The lines were also present in the cortex below the cut. The fungus was recovered in culture from the wood. The controls were unaffected after ten days.

Nos. 8, 9 & 10—Similar to above but a suspension of zoospores was used and applied to the tapping cuts on wet cotton wool. All were positive.

No. 11—Untapped virgin bark was lightly scraped over an area of one inch and inoculated with mycelium and zoospores on a piece of wet cotton wool. In twelve days a patch of diseased tissue had formed measuring $2\frac{1}{2}$ x $1\frac{1}{4}$ inches. The patch was brownish pink in colour in the outer cortex, and reddish brown in the inner cortical layers and the outer layers of the wood, and was bounded by a greenish black, irregular line. The fungus was reisolated.

No. 12—The experiment was repeated on twenty trees of which ten were unwounded. All the wounded trees and four of the unwounded trees gave a positive result, the wounded trees after a few days, and the unwounded trees in from eight to ten days. The patches were very similar in all cases. Three control trees were unaffected.

No. 13—Two young papaya fruits (*Carica papaya* L.) were wounded and inoculated. There was no result until the eleventh day when a little mycelium was produced. The fruits rotted in fourteen days.

No. 14—Two plants of *Colocasia antiquorum* were inoculated on the leaves and at ground level. The result was negative even when the leaves were wounded.

No. 15—Two young Castor Oil seedlings were inoculated by wounding at soil level and pouring a suspension of zoospores over the wounds. After ten days there was

no result, so the plants were reinoculated, using mycelium and zoospores on moist cotton wool which was placed in contact with the stems of wounded and unwounded plants. The wool was kept moist for two days. The result was positive, all the plants wilting in eight to ten days. The fungus was reisolated. Controls were unaffected.

No. 18—Two plants of *Hibiscus sabdariffa L. var. altissima* (Roselle fibre) were inoculated at the collar. One was wounded and one was unwounded. The result was negative.

No. 19—Two potato tubers were inoculated with the fungus. The potatoes were sterilised by soaking for three minutes in corrosive sublimate (1 : 1000) and washed in sterile water. They were then sliced and put in sterile glass jars. One of the slices in each jar was inoculated with mycelium. The result was positive, the slice being rotted in six days. A whole tuber was also inoculated and kept in a damp chamber. In five days sporangia and chlamydospores were formed in spots on the surface. The flesh of the tuber, although moderately firm in the centre, was rotted near the surface, and was full of hyphae.

No. 20—Two young tobacco plants were inoculated with the fungus at the collar, and on the leaves. The result was negative.

No. 21—Two young tomato plants were inoculated on the stem, and at soil level. The result was negative.

No. 22—Two young coconut palms were inoculated by boring a hole through the leaf bases just above the bud. A potato plug, on which the fungus was growing vigorously, was inserted and the hole was plugged. After a year there was no result. The leaves had grown and carried the fungus away from the bud. There was no rot in the leaf bases.

The results of these inoculations are summarised in Table 2 (+ ve = positive; — ve = negative).

TABLE 2.

Host plant.	Wounded or unwounded.	Point of inoculation.	Result.	No. of days after inoculation
Hevea Brasiliensis Muell. Seedlings	W	Collar	– ve	90
do do ...	W	,,	+ ve	30 Plant was not killed
Hevea Brasiliensis Muell. Leaves	W & U	midrib and epidermis	– ve	14
do do ...	,,	,,	– ve	10
Hevea Brasiliensis Muell. Fruit	W	Near the stalk end	+ ve	4–7
do do ...	U	,,	+ ve	7
Hevea Brasiliensis Muell.	W	Tapping cut	+ ve	3
do do ...	W	Virgin Bark	+ ve	12
do do ...	U	,,	+ ve	8–10
Carica papaya L. ...	W	Fruit	+ ve	11
Colocasia antiquorum Sch.	W & U	Leaves and collar	– ve	30
Riccinus communis L.	W & U	Stem at soil level	+ ve	8–10
Hibiscus sabdariffa L. var. altissima	W & U	Collar	– ve	21
Solanum tuberosum L.	W	Tuber	+ ve	5
Nicotiana tabacum L.	W	Collar	– ve	30
Lycopersicum esculentum Mill.	W	Stem and collar	– ve	30
Cocos nucifera L. ...	W	Leaf bases	– ve	365

Mixed Cultures.

In 1922, Ashby (5) published a paper on the oospores of *P. faberi Maubl.* in which he announced the finding of oospores in cultures when two strains of this fungus were grown together. In 1924, Gadd (6) published a paper on *P. faberi* Maubl. in which he distinguishes two groups of this

fungus, a rubber group and a cacao group. When a strain of the first group is grown in culture with a strain of the second group, oospores are produced, but they are not formed when members of the same group are grown together.

The Black Stripes species No. 1 is considered to be a member of the rubber group of *P. palmivora* Butl., owing to the mean ratio of the length to the width being greater than 1.50. Further, when grown in mixed culture with members of the cacao group, viz:—No. 11 *P. palmivora* Butl. (coconut, India), and No. 20 *P. palmivora* Butl. (Cacao, Trinidad), oospores are formed readily. Oospores were not obtained with No. 20 when grown in Malaya, but they developed when the mixture was made at Kew. No. 20 died out soon after its arrival in Malaya, as it was not vigorous, and this probably influenced the result. Oospores were absent in mixed cultures with members of the rubber group. Quaker Oats, Maize, and Green Pea Agars form suitable media for oospore production. On the first two, oospores turn yellow, but they remain clear for some time on Green Pea Agar. On this medium the mean diameter of 25 oospores was 23 microns.

No. 2 Phytophthora palmivora Butl. obtained from Black Stripe of Hevea in Chemor, Perak, (1928).

This fungus was isolated from the wood of a diseased tree from an area in which Black Stripe was very prevalent. In culture the growth is similar to that of No. 1; sporangia and chlamydospores being formed readily on all media used.

Quaker Oats Agar.

On this medium there is a vigorous growth of aerial mycelium, and chlamydospores are formed in two days. At first chlamydospores are found in greater abundance than sporangia which do not begin to appear in any numbers until the fourth day.

Maize, Green Pea, and Potato-dextrose Agars, and Potato Blocks in Roux tubes.

On these media the type of growth is similar to that described for species No. 1.

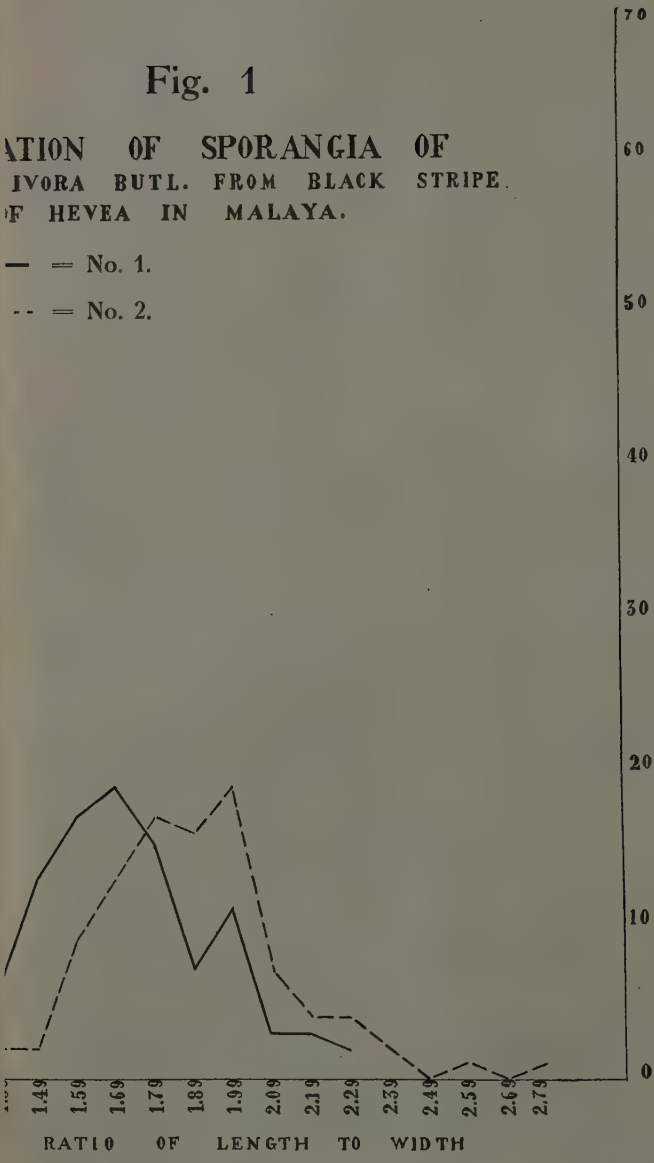
70
60
50
40
30
20
10
0
37.49
41.49
45.49
49.49
53.49
57.49
61.49
65.49
69.49
73.49
77.49
LENGTH IN MICRONS
21.49
25.49
29.49
33.49
37.49
WIDTH
IN MICRONS
1.09
1.19
1.29
CLASS
VARI
P. PALM

Fig. 1

ATION OF SPORANGIA OF
IVORA BUTL. FROM BLACK STRIPE
F HEVEA IN MALAYA.

— = No. 1.

-- = No. 2.



The fungus is of the same species as No. 1, but the sporangia have more elongated papillae and are not so wide on the average. Otherwise its morphology is similar, and, when inoculated into *Hevea*, Castor, Potato and Papaya, it gives a similar effect. One curious feature of the fungus is that a number of sporangia, on germinating, produce a short germ tube on which a chlamydospore is formed. When the sporangium becomes free, if placed in water, the chlamydospore remains attached and the two float about together. (Plate I).

Measurements of the sporangia have been obtained on Maize Agar. Details are given in Table 3.

TABLE 3.

Sporangia of No. 2 (P. palmivora Butl.), obtained from Black Stripe of Hevea Chemor, Perak, F.M.S.

Medium	Number measured	Range in microns.	Mean in microns	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
Maize Agar ..	100	36-75 x 21-36	50.97 x 26.93	1.21-2.79	1.87

This strain is very typical of the rubber group of *P. palmivora* Butl. and in mixed culture it behaves similarly to No. 1, forming oospores when mixed, on Green Pea Agar, with No. 11. The mean diameter of these oospores was 24.16 microns.

The variation in the length etc. of the sporangia of No. 1, and of No. 2, on Maize Agar, is illustrated graphically in Fig. 1.

No. 3 PHYTOPHTHORA SP. OBTAINED FROM BLACK STRIPE OF HEVEA, IN NEGRI SEMBILAN, F.M.S.

This species was isolated from a bark specimen received from Seremban.

Quaker Oats Agar.

On this medium a strong growth of mycelium is formed. The growth is dense and fills the test tube with a white,

compact mass of mycelium in seven to eight days. No sporangia have been found, either on the aerial mycelium, or in the medium, until after twelve days. As the culture ages a moderate number are formed.

Maize Agar.

On this medium the growth is very similar to that on Quaker Oats Agar.

Green Pea Agar.

Mycelial growth is good but not so dense as on the above media. The sporangia, which are produced in fair numbers after eight days, are of two types—broadly ovate with papillae and spherical with no papillae.

Potato dextrose Agar.

Mycelial growth is good. At first it is confined to the surface of the agar along which it spreads in a dense fan of "parallel" hyphae. Aerial hyphae are formed behind the advancing edge of growth and form a compact smooth mass. The sporangia which develop after eight days are small.

One of the best methods of obtaining large numbers of sporangia is to grow the fungus in water to which a seed of *Vigna catjang* is added. A green pod is opened and a young bean extracted with a sterile needle. This is placed in a little sterile water in a sterile petri dish, and mycelium from a young culture is placed on the bean. After twenty four hours a good growth of mycelium is obtained in the water surrounding the bean, and in three days thousands of sporangia are produced. These sporangia tend to be larger and fatter than those produced in agar culture. A variety of shapes are formed but the majority are broadly ovate, with rounded papillae. Some appear almost circular and have papillae which are only seen by careful focussing. Some are circular and have no papillae. They are slightly smaller than the circular, papillate sporangia, but are not quite as thick walled as chlamydospores of the "palmivora" type. Loose sporangia may or may not have a portion of the sporangiophore attached. Species No. 6, and No. 7 (*P. meadii* McRae) form similar large numbers and types of sporangia and "sphaeroconidia" if grown in this water medium.

Potato Block in Roux Tubes.

The fungus grows slowly but in ten days produces a little aerial mycelium. A few sporangia are formed about the fourteenth day, both types being present. The round bodies (27—34 microns) resemble chlamydospores, but they are not definitely thick walled.

Malt Extract Agar. (Leonian's formula).

After six days there is a slight growth of aerial mycelium. A few sporangia are produced about the fourteenth day.

Glucose Meat Extract Agar.

The growth is slow and mostly submerged but a little powdery surface mycelium is formed. Both types of sporangia are present in seven days but they are very few in number.

Measurements of the sporangia have been obtained from Green pea Agar, Water and bean, and Quaker Oats Agar and are given in Table 4. The circular forms, without papillae have not been included.

TABLE 4.

Sporangia of No. 3 Phytophthora sp. from Black Stripe of Hevea, Negri Sembilan, F. M. S.

Medium	Number measured	Range in microns.	Mean in microns	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
Green Pea Agar	100	26-51 x 20-39	36.50 x 28.17	1.08-1.85	1.34
Water & Bean ...	100	33-69 x 27-42	47.01 x 33.69	1.08-2.30	1.44
Quaker Oats Agar	100	30-66 x 21-39	41.67 x 30.29	1.08-2.44	1.37

INOCULATION EXPERIMENTS.

No. 1—Untapped *Hevea* bark was wounded, and inoculated in the same way as described for species No. 1. The result was positive after 10 days; a patch 2″ x 3½″ had formed in the bark, while the wood was penetrated to a depth of ½ an inch.

No. 2—Untapped and unwounded *Hevea* bark was inoculated. The result was positive after ten days. The patch formed was smaller than in No. 1 experiment, but the infection had reached into the wood and had penetrated it slightly. The fungus was reisolated.

No. 3—A tobacco plant, one foot in height, was wounded at soil level and inoculated with a suspension of zoospores and mycelium. In five days the plant wilted. The roots were found to be in an unhealthy condition but the stem tissues were not affected. Attempts to reisolate the fungus were not successful. The control also became infected and died, so the experiment was inconclusive. The experiment was repeated on two healthy plants and the result was negative.

No. 4—The tapping cut of a rubber tree was inoculated with the fungus. Black Stripe developed in a few days.

No. 5—A pod of *Hevea* was slightly wounded and inoculated with mycelium and zoospores and kept moist. In six days the pod was rotted and covered with mycelium.

No. 6—Three unwounded pods of *Hevea* were inoculated with mycelium and zoospores. The pods, which were attached to a cut branch standing in water, were covered with a bell-jar containing moist cotton wool. The inoculum was placed in a drop of water around the stalks of the pods. The water did not evaporate for two days but there was no sign of rot. After seven days the pods were still green and fresh.

No 7—The above pods were pricked with a needle until latex flowed, and a fresh inoculation was made near the wounds. In two days a watery discoloration appeared near the wounds, and, four days later, the pods were covered with mycelium and rotted.

No. 8—Two plants of Roselle (*Hibiscus sabdariffa* L.) were inoculated at soil level; both were slightly wounded. The result was positive after eight days.

No. 9—Two Castor Oil plants were wounded and inoculated at soil level. The plants wilted in six days and the fungus was recovered from the stem.

No. 10—Two potato tubers were inoculated. A wet rot appeared after six days, but no external mycelium was formed. Controls remained normal.

No. 11—Two young *Hevea* plants were wounded slightly, and inoculated at the collar, and on the stem three inches from soil level. After a month there was no sign of disease externally, but, on cutting open the bark under the inoculations, it was found that the bark and wood were discolored with blackish green lines which extended about two inches up and down the stem. The collars of the seedlings were not quite girdled by the fungus nor was the stem girdled higher up. The wood tissue was full of hyphae which had not quite penetrated to the pith. The leaves looked quite healthy when compared with the controls. The fungus was reisolated from the wood.

Mixed Cultures.

Oospores have not been formed in pure culture, but, if the fungus is grown in mixed culture with No. 8 (from *Hibiscus sabdariffa* L.) , oospores, with amphigynous antheridia, are formed after ten days. No. 8 does not form oospores in pure culture, but it is a species distinct from No. 3, and bears a close resemblance to No. 13 (*P. parasitica* Dast.) Its taxonomy will be discussed later. Oospores also are formed in abundance when No. 3 is grown in mixed culture with No. 12 (*P. arecae* Coleman); they appear in four or five days. *P. meadii* McRae and *P. arecae* Coleman, are considered by Ashby (7) to be very similar and possibly strains of one species. They have not formed oospores in pure culture in Malaya, but if grown together in mixed culture, oospores, with amphigynous anteridia, are freely formed in five days on Green Pea Agar. They range from 17—27 microns, the mean of 50 being 24.32 microns. The oospores formed when No. 3 is mixed with *P. arecae* range from 21—28.6 microns, the mean being 24.70 microns. Oospores have not been formed, after a month, when No. 3 was grown, in mixed culture, with No. 7 (*P. meadii* McRae) on Green Pea Agar.

This suggests a relationship between No. 7 (*P. meadii* McRae) and No. 3, which is further confirmed by the fact that oospores are formed after ten days on Green Pea Agar, if No. 7 (*P meadii* McRae) is grown in mixed culture with

No. 8 (from *H. sabdariffa* L.) They range on this medium from 18.5—27 microns, the mean of 50 being 23.04 microns. The oospores formed in the mixture of No. 3 with No. 8 range from 19—28 microns the mean being 23.80 microns.

The sporangia of No. 7 (*P. meadii* McRae) are slightly larger than those of No. 3, but the length to width ratio is the same (1.32 and 1.34 on Green Pea Agar) (Fig. 2). The sporangia of No. 12 (*P. arecae* Coleman) are nearer in size to those of No. 3, but the length to width ratio is slightly smaller (1.28 and 1.34).

The mycelium and type of growth on various media, is very similar for all three strains.

If No. 7 (*P. meadii* McRae) and No. 12 (*P. arecae* Coleman) are considered to be strains of one species, then No. 3 can be included in this species, under the name—for the present—of *P. meadii* McRae, since it behaves similarly to that species in mixed culture, and has a similar morphology. The oospores formed when No. 3 and No. 7 are each grown with No. 8 in mixed culture, are not formed until the tenth day, while in the mixtures with No. 12 (*P. arecae* Coleman) they appear in abundance after the fourth day. *P. meadii* McRae is known occasionally to form oospores in pure culture in India, and in the mixtures with a distinct species (No. 8), oospores are also formed; but it is uncertain to which of the two species they belong, since there is no very significant difference in size between these oospores and those formed in the *meadii*, and *arecae* mixtures, and mixtures of No. 8 with strains of No. 13, and No. 16 (*P. parasitica* Dast), and Nos. 1 and 2 (*P. palmivora* Butl. rubber group). (See page 87).

No. 4 Phytophthora sp. obtained from Black Stripe of Hevea, in Malacca, 1928.

The fungus was isolated from the wood of a tree affected with Black Stripe. The affection was slight, only two thin stripes being evident. The tree was growing in a native holding and was apparently the only one attacked by this disease. Almost all the other trees in tapping were badly affected with Mouldy Rot, but in no case were the two diseases found on one tree. Three days after the culture was prepared, large numbers of oospores, with amphigynous antheridia, were

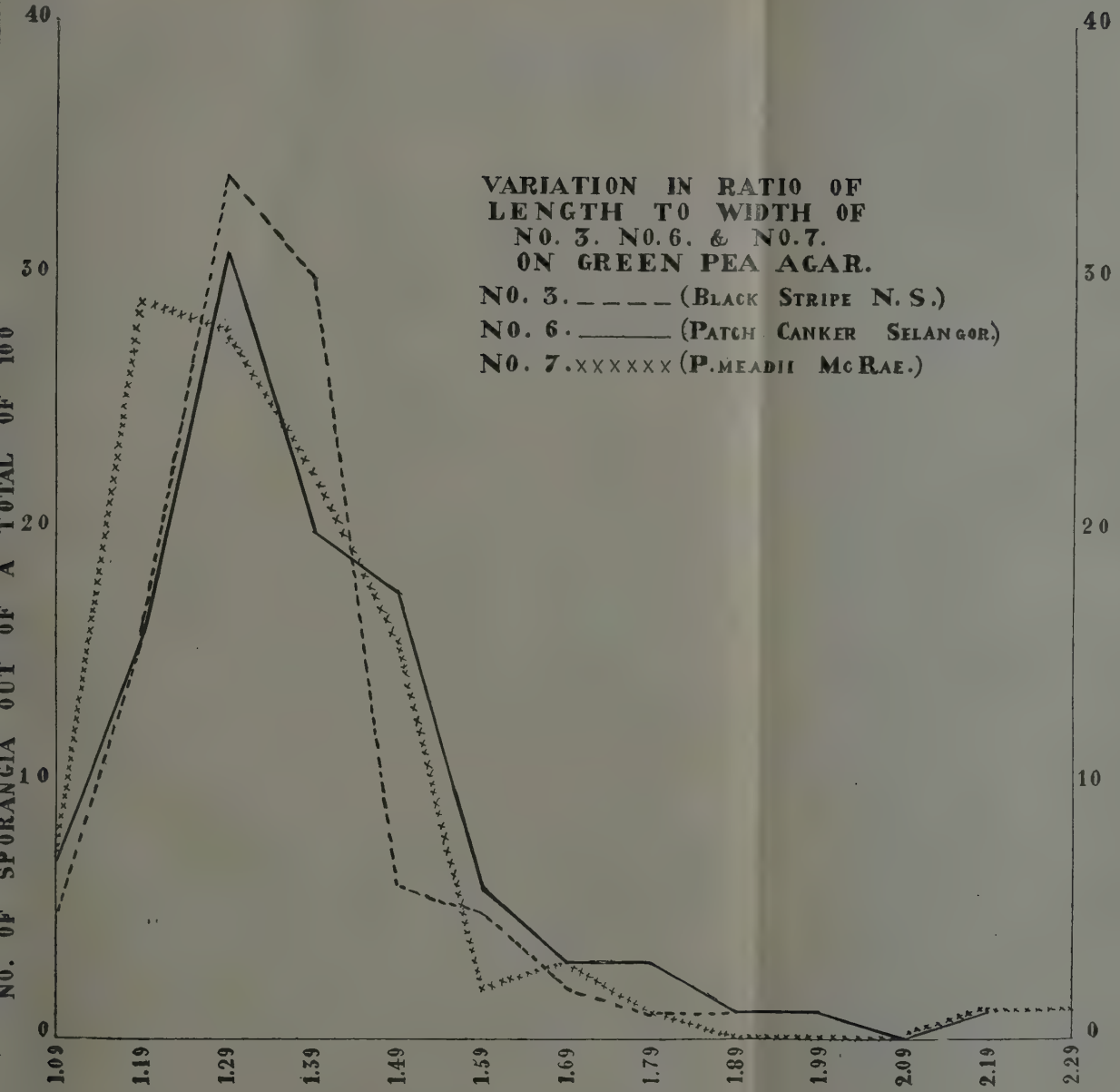

VARIATION IN RATIO OF
LENGTH TO WIDTH OF
NO. 3. NO. 6. & NO. 7.
ON GREEN PEA AGAR.
NO. 3. _ _ _ _ (BLACK STRIPE N. S.)
NO. 6. _____ (PATCH CANKER SELANGOR.)
NO. 7. xxxxxx (P. MEADII McRAE.)
NO. OF SPORANGIA OUT OF A TOTAL OF 100
40
30
20
10
0
1.09
1.19
1.29
1.39
1.49
1.59
1.69
1.79
1.89
1.99
2.09
2.19
2.29
CLASS RATIO OF LENGTH TO WIDTH

FIG. 2.

formed on the surface of the medium. A few sporangia had formed, and these were present in large numbers on the following day. It was soon evident that the fungus was identical with No 5 which had been isolated from *Hevea* pods in the previous year.

No.5 Phytophthora sp. obtained from Pod Rot of Hevea, in Selangor, 1927.

In December 1927, some pods of Hevea which were rather late in forming, fell from a tree while still green. The pods were discolored with dark, watery, green patches, some near the stalk end and others at the opposite end. Cultures were prepared and freed from impurities in the usual way.

The fungus in culture.

Quaker Oats Agar.

In two days there is a good surface growth of mycelium, but aerial mycelium is not produced until after fourteen days, and even then it is very scanty. The first cultures from the pods produced oospores in large numbers, but only a few sporangia until the seventh day. In subsequent transfers however, sporangia developed early. They are irregular in shape. No definite chlamydospores are formed, but some of the spore forms are circular. The sporangiophore is frequently inserted laterally.

Green Pea Agar.

No aerial mycelium is formed on this medium, but the surface growth is good. Sporangia are formed in two days on the surface and in the medium; they are produced in abundance and are very irregular in shape. Some are broadly ovate; some are circular with no papillae; others are long and narrow with prominent papillae, obpyriform, or curved. Oospores appear after the fourth day in large numbers, both on the surface and in the medium.

Potato Agar.

The fungus does not grow well on this medium; there is no aerial mycelium formed and the mycelium in the medium

is scanty, but the hyphae are larger than usual. Oospores are formed, but they are very few in number. A large number or oogonia with antheridia develop, but the majority disintegrate without forming oospores. No sporangia are formed up to three weeks.

Potato dextrose Agar.

On this medium there is sometimes a slight aerial growth which is rather tufted. The medium becomes cloudy and wrinkled (bacteria were proved to be absent). Sporangia are sparingly produced at first, and large numbers of oospores develop in the medium.

Maize Agar.

Scanty aerial mycelium may be formed on this medium, but the surface growth is good. Sporangia and oospores are formed in large numbers. The majority of sporangia are of the more narrowly ovate type, and many of them have the sporangiophore at the side.

Potato Blocks in Roux Tubes.

There is no growth on this medium.

Malt Extract Agar. (Leonian's formula).

Growth is entirely submerged. Large numbers of sporangia are produced after two days. They are very misshappen, some are curved and distorted, others are long and narrow and rounded at both ends, having no papillae. A few are roughly circular, thick-walled, types with no papillae. No oospores are formed up to the fifth day, but large numbers are present by the fourteenth day. Many are coloured yellow brown.

Water and Bean.

In two days a fair quantity of mycelium is present. Sporangia and oospores are formed on the fourth day. The sporangia are more regular in shape than those on solid media some are broadly ovate, and some are almost spherical. A number of the sporangia look like chlamydospores but they are rather thinner walled than these bodies, and many, which appear to be circular at first, shew papillae when focussed

more carefully. The elongated forms are absent, or few in number, in the earlier stages of growth. Measurements of sporangia and oospores are given in Table 5.

TABLE 5.

Sporangia and oospores of No. 5 Phytophthora sp. from Hevea pods, in Selangor, F.M.S.

Sporangia.

Medium	Number measured	Range in microns	Mean in microns	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
Potato-Dextrose Agar ...	100	27-63 x 20.5-42	44.31 x 27 89	1.12-2.48	1.56
Maize Agar ..	100	30-60 x 21-39	45.85 x 27.42	1.09-2.85	1.73
Quaker Oats Agar	100	30-66 x 21-48	45.06 x 31.13	1.05-2.57	1.47
Green Pea Agar	100	36-60 x 21-39	46.74 x 32.07	1.15-2.14	1.46
Water & Bean ...	100	36-44 x 27-36	39.13 x 33.13	1.08-1.33	1.17

Oogonia & Oospores.

Medium	Number measured	Range of oogonia in microns	Range of oospores in microns	Mean of oogonia in microns	Mean of oospores in microns
Quaker Oats Agar	100	17-30	15-24	25.25	20.43
Green Pea Agar ...	50	21-28	18-24	24.76	20.17
Maize Agar ...	50	—	18-26	—	22.00
Potato-Dextrose Agar	50	21-32	17-26.8	27.74	23.25

Inoculation Experiments.

No. 1—The tapping cuts of two *Hevea* trees were inoculated with mycelium and zoospores. The trees were tapped daily, and, in four days, shewed signs of Black Stripe. The fungus spread downwards into the wood below the cut, and, near the cut, penetrated into the wood to a depth of half an inch.

No. 2—A little Agar medium containing spores and mycelium of the fungus was smeared over the bark of a

tree in tapping just above the cut. In five days definite Black Stripe symptoms were present.

No. 3—The leaves and petioles of a growing rubber seedling were inoculated, by placing a little agar medium, containing mycelium and zoospores, on the leaves and at the junction of the petiole and the stem. The leaves and stem were pushed into a flask containing wet cotton wool. The inoculated leaves fell in two days but no rot was noticed.

No. 4—Young and old leaves of a rubber seedling were inoculated as above, but the leaves were folded to retain a small drop of water. Zoospores were placed in the water drops which did not evaporate for two days. After six days a watery spot, less than half an inch in diameter, developed on some of the young leaves. Hyphae were present in the spots, but attempts to re-isolate the fungus were unsuccessful, probably owing to the large numbers of protozoa and bacteria which developed in the cultures. The older leaves were not affected.

No. 5—The above experiment was repeated but only one leaf became diseased.

No. 6—Two *Hevea* seedlings were wounded and inoculated at soil level. The result was similar to that of experiment No. 11 with fungus No. 3. The seedlings were not killed after a month, but the fungus was present in the stem and was reisolated at the end of this period.

No. 7—Six *Hevea* pods were sterilised with corrosive sublimate, washed in sterile water, put into sterile, moist jars, and inoculated at the stalk end. Three pods were wounded, two were unwounded, and one wounded pod was kept as a control. In six days the wounded pods were covered with mycelium, and a little mycelium was present on the unwounded pods, which were discolored near the stalk. On the eighth day these stalks became covered with a mixture of *Gloeosporium* sp. and *Phytophthora*. The control developed *Gloeosporium* on the tenth day (probably introduced by mites). Oospores were found in the mycelium on the pods; they were yellowish in colour,

No. 8—Castor Oil seedlings were killed by the fungus in four days.

No. 9—Untapped *Hevea* bark was wounded and inoculated with mycelium and zoospores. In fourteen days a patch had formed in the bark and wood. Oospores were found in the tissues of the wood.

No. 10—Potato tubers were sterilised in corrosive sublimate, wounded and inoculated, and kept in sterile jars. The result was negative.

No. 11—A tobacco plant was wounded and inoculated on the stem above soil level. The result was negative.

No. 12—An immature papaya fruit was wounded and inoculated and kept under a bell jar. The fruit remained healthy for twenty days after which it slowly decayed. The control gave a similar result, and, since the original fungus was not recovered from the inoculation, the result must be considered negative.

Description.

Mycelium.—The mycelium is non-septate when young, but in cultures of a month old, septa are frequently formed. The hyphae are but slightly branched and are rarely wider than 5 microns, the average being about 4.6 microns. Aerial mycelium is absent or very scanty. A little may be formed on Maize Agar after fourteen days but sometimes none is formed.

Sporangia.—These are of several types and vary greatly in shape on Agar media. The papillae are sometimes pointed and sometimes round. The sporangiophore is very frequently inserted at the side, especially in the case of curved sporangia. In a few instances there is a swelling in the sporangiophore some distance beneath the sporangium and immediately above this swelling there may be a septum. Spherical. papillate sporangia, sometimes with thicker walls than normal, are common, and in some cases the papillae are not very evident. These types look like chlamydospores, but if the cover slip is moved carefully, some of them turn over and papillae can then be seen. No true chlamydospores, either intercalar or terminal, have been found.

Oospores.—These bodies are produced early and in large numbers, on all the Agar media, and in water culture. The oogonium is characteristically "funnel" shaped and is surrounded at the base by the antheridium through which it appears to have penetrated. Paragynous antheridia have not been seen. The oospores frequently almost fill the oogonial cavity, and have fairly thick, smooth walls (about 3 microns wide). They are not definitely coloured on Green Pea Agar, but in one month old cultures on Maize Agar they are sometimes brownish, and on Malt-Extract Agar the majority are slightly yellowish. The contents are granular and oily; a vacuole is frequently present.

Taxonomy.—The fungus has not been previously reported from Malaya, and it differs from No. 7 (*P. meadii* McRae) which causes *Hevea* pod-rot in South India. The absence of aerial mycelium, the shape of the oogonium, the smaller and more numerous oospores, and the larger length to width ratio of the sporangia of the Malayan species, are sufficiently significant characters to separate it from No. 7 (*P. meadii* McRae). The absence of chlamydospores, and the non-production of aerial mycelium, separates the species from Nos. 13 and 16 (*P. parasitica* Dast.), and Nos. 9 and 10 (*P. colocasiae* Rac), Nos. 1, 2, 11, 18, 19 and 20 (*P. palmivora* Butl.), and Dastur's species from *Hevea* in Burma (8). Significant differences also exist between the Malayan fungus and No. 21 (*P. cinnamomi* Rands), No. 12 (*P. arecae* Coleman), *P. erythroseptica* Pethyb. (N.L.), *P. infestans* Mont. de Bary (N.L.), and *P. Jatrophae* Jens. (N.L.).

The exclusively amphigynous antheridia and other characters separate it from *P. cactorum* Cohn and Lebert. (N.L.), *P. fagi* Hartig (N.L.), *P. syringae* Kleb. (N.L.), *P. Paeoniae* Cooper & Porter (N.L.), *P. pini* Leonian (N.L.).

Rosenbaum (9) established a *Phaseoli* group in his classification of the *Phytophthorae*, and the Malayan species would be included in this group from the fact that the antheridium is at the base of the oogonium. The members of this *Phaseoli* group are *P. phaseoli* (N.L.), *P. erythroseptica* (N.L.), No. 12 (*P. arecae*), No. 13 (*P. parasitica*), and *P. infestans* (N.L.). Of these species *P. phaseoli* is perhaps nearest to the local species, but the sporangia are stated to be small, (27.87 x 19.05 microns) and fluffy aerial mycelium is formed on Oat, Maize, and Bean Agars. On Malt Extract Agar *P. phaseoli*,

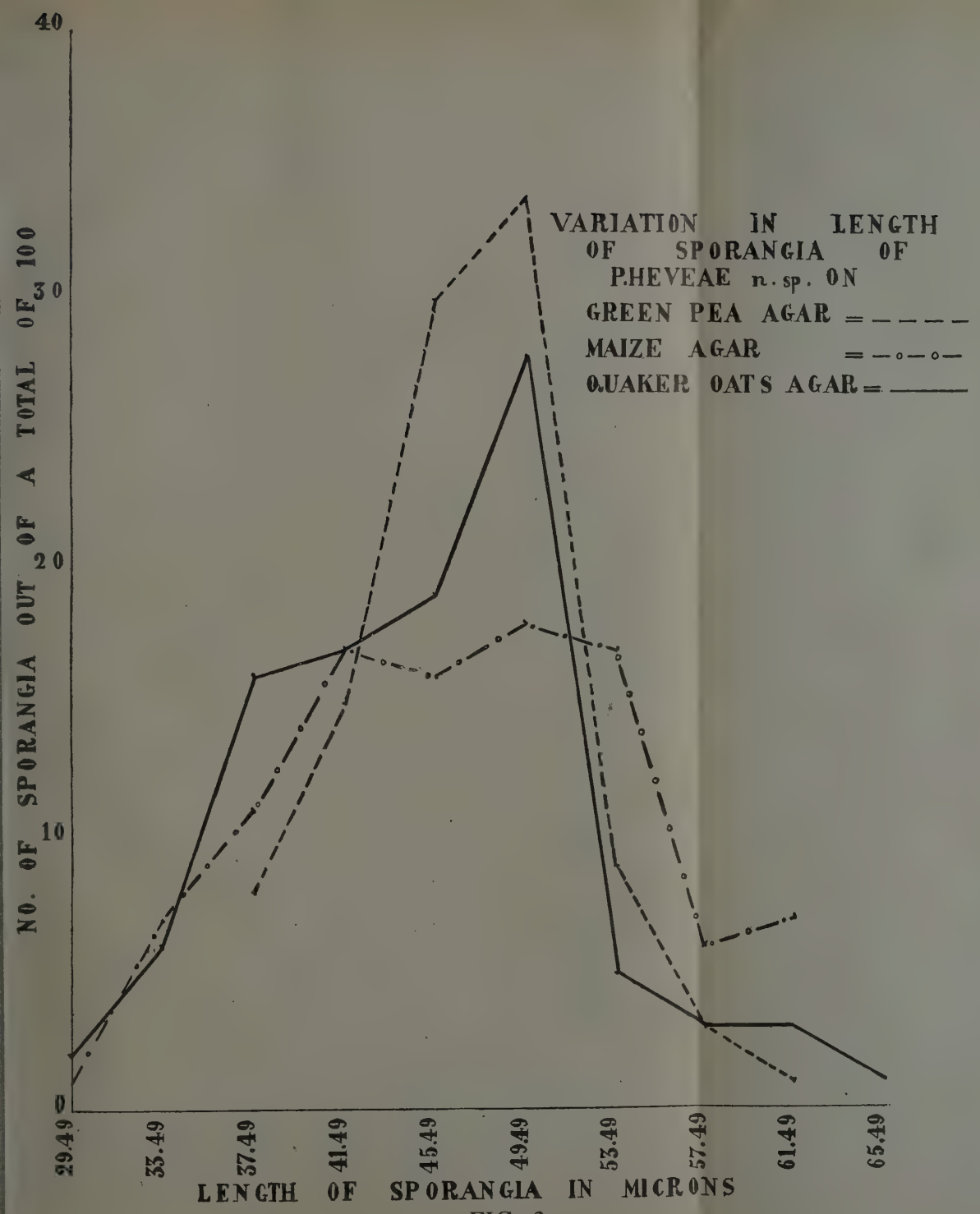

VARIATION IN LENGTH OF SPORANGIA OF PHEVEAE n. sp. ON
GREEN PEA AGAR
MAIZE AGAR
QUAKER OATS AGAR
NO. OF SPORANGIA OUT OF A TOTAL OF 100
40
30
20
10
0
29.49
33.49
37.49
41.49
45.49
49.49
53.49
57.49
61.49
65.49
LENGTH OF SPORANGIA IN MICRONS

FIG. 3.

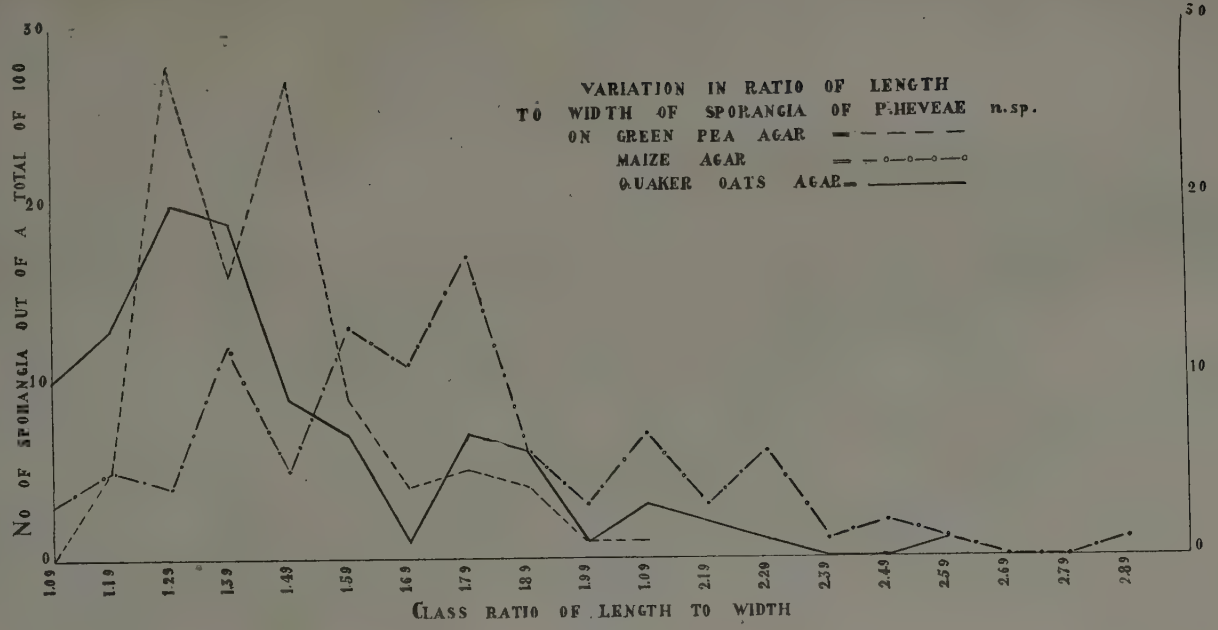

VARIATION IN RATIO OF LENGTH
TO WIDTH OF SPORANGIA OF P. HEVEAE n. sp.
ON GREEN PEA AGAR
MAIZE AGAR
QUAKER OATS AGAR
No OF SPORANGIA OUT OF A TOTAL OF 100
0
10
20
30
1.09
1.19
1.29
1.39
1.49
1.59
1.69
1.79
1.89
1.99
2.09
2.19
2.29
2.39
2.49
2.59
2.69
2.79
2.89
CLASS RATIO OF LENGTH TO WIDTH

FIG. 4.

according to Leonian (10), forms but few sporangia, moderate aerial mycelium, and does not form a colony. The *Hevea* species forms a colony, 35 mm. in diameter, in two days at 25°—30°C., and produces large numbers of sporangia and no aerial hyphae.

These differences are considered to be sufficiently significant to separate the *Hevea* species from *P. phaseoli* Thaxter, and it is proposed to regard it as a new species, and to name it *P. heveae* (Thompson). The species has been examined by the Mycologist (S. F. Ashby) at the Imperial Bureau of Mycology who states "It has not been possible to refer this to any known species". The description is as follows:—

Phytophthora heveae n.sp.—Mycelium submerged on Agar media, and not formed on Potato Blocks, non-septate when young, 2-7 microns in width. Sporangia present on most Agar media, and in water culture to which a seed of *Vigna catjang* is added, but absent or few in number on Potato Agar; somewhat irregular in shape, varying from spherical to narrowly ovate; sporangiophore frequently inserted laterally. Range of sporangia 27-66 x 20.5-48 microns, average of 400 = 45.49 x 29.62 microns. Mean ratio of length to width = 1.55. Oogonia and oospores freely formed on Potato-dextrose and other Agar media and also in water. Oogonia broadly funnel shaped with amphigynous antheridia. Oospores round, smooth, and thick walled, not always coloured distinctly. Range of 200 oogonia = 17-32 microns, mean = 25.91 microns, range of 250 oospores = 15-26.8 microns, mean = 21.46 microns. Parasitic in Malaya on bark and fruit of *Hevea brasiliensis* (H.B.K.) Muell. Arg. Para Rubber.

No 6, Phytophthora sp. isolated from Patch Canker of Hevea brasiliensis in Selangor F.M.S. (1926).

The fungus was obtained from the bark of a ten year old rubber tree which had never been tapped. The tree was one of three in a group and all were diseased, having one or more patches of discolored bark about four feet from the ground. The patches were not larger than roughly 2-3 inches in diameter and the disease had penetrated into the wood. The fungus was isolated from the inner cortex which was greyish red in colour.

Growth in Culture.

Quaker Oats Agar.

After five days there is a copious growth of somewhat lanate aerial mycelium. Sporangia are sparingly produced until after the ninth day when the number increases.

Maize Agar.

The growth is similar to that on Quaker Oats Agar.

Green Pea Agar.

A fair amount of aerial mycelium is present in three days. Sporangia may be present on the fourth day and are numerous after twelve days.

Potato dextrose Agar.

After five days there is copious aerial mycelium present. Sporangia are present after the seventh day. Growth on this medium is similar to that of No. 3 on the same medium.

Potato Agar.

Growth is mostly in the medium, aerial hyphae being scanty. No sporangia up to the fourteenth day.

Potato Blocks in Roux Tubes.

The fungus grows slowly on this medium, but in time a quantity of aerial mycelium is formed. Sporangia are not formed until after a month.

Baked Slices of Young Papaya Fruit.

There is a strong growth of aerial mycelium, but no sporangia until after fourteen days and then only a few develop.

Water and Bean.

In two days the water contains a good growth of mycelium in groups, probably formed by zoospores liberated from

sporangia in the inoculum. In three days large numbers of sporangia are present.

Malt Extract Agar.—(Leonian's formula). Growth on this medium is similar to that of No. 3 and No. 7 (*P. meadii* McRae), on the same medium. Mycelium is sparsely aerial in six days, and sporangia few in number on the fourteenth day. The number increases after a month but they are not numerous.

Description.

Mycelium.—This is produced copiously on most media. The aerial hyphae are little branched and range from 1.5—6 microns in diameter. The submerged mycelium is more branched and the contents more granular. The hyphae range from 2—9 microns in diameter.

Sporangia.—These are regular in shape, curved types being rare. The majority are inclined to be somewhat broadly ovate, (l/w ratio less than 1.50) and have prominent papillae. The sporangiophore is usually at the base, rarely lateral, and part of it may remain attached to the free sporangia. No definite chlamydospores have been found, but circular spore forms are developed. Some of these have papillae which are often difficult to see, but in some no papillae can be distinguished. These types were, at first considered to be chlamydospores, but they are not so thick walled as the chlamydospores of No. 1 (*P. palmivora* Butl.) or No. 12 (*P. parasitica* Dast.) They range from 27-42 microns in diameter, and are probably similar to those forms found in species No. 3, No. 5 and No. 7, and to the reduced sporangia which were described in the species obtained from Black Stripe in 1916 (2). A few elongated, narrowly ovate, sporangia, are also developed, especially on Maize Agar, and in old water and bean cultures. In the latter medium sporangia are very numerous; at first they are broadly ovate and resemble those of No. 3 and No. 7 (*P. meadii* McRae), but later on distorted forms sometimes appear which are very long and narrow. Circular forms with no papillae are fairly numerous.

Zoospores.—The sporangia when placed in water germinate directly, or may produce zoospores. The large sporangia do not often produce zoospores, but the smaller ones do so, soon after they are placed in water. No difficulty has been

experienced in obtaining zoospores, from sporangia which have developed in cultures grown purely on artificial media for long periods. They are produced in the usual manner, escaping one by one or in groups, from the opening in the papillae of the sporangia.

Oospores.—No oospores have been formed in pure culture on any medium.

Measurements of sporangia have been obtained from cultures soon after the appearance of the sporangia. The circular forms without papillae have not been included, as they are regarded as being reduced sporangia.

TABLE 6.

Sporangia of No. 6 Phytophthora sp. isolated from Patch Canker of Hevea, in Selangor, F.M.S. 1926.

Medium	Number measured	Range in microns.	Mean in microns	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
Potato-Dextrose Agar ..	200	27-69x21-45	41.57x30.64	1.10-1.82	1.36
Maize Agar	100	33-66x24-39	42.08x29.60	1.11-2.50	1.45*
,, ,, ...	100	27-63x21-42	43.08x30.96	1.10-2.33	1.39
Quaker Oats Agar	100	33-66x27-45	46.98x33.67	1.13-2.22	1.39
Green Pea Agar	200	27-72x21-45	43.85x27.38	1.02-2.20	1.36
Water & Bean ...	50	36-66x24.42	46.00x32 15	1.18-1.80	1.44

*fresh isolation.

Inoculation Experiments.

No. 1—Two Hevea seedlings, two months old were wounded and inoculated, with mycelium and zoospores (a) at the collar, (b) on the stem three inches from the ground, (c) on the leaves and petioles. All were kept moist with cotton wool.

Some of the inoculated leaves fell after a week, but there was no discoloration. The tissues of the stem

and collar were discolored similarly to those of the seedlings inoculated with species No. 1, No. 3 and No. 5, but the plants were not killed. The fungus was recovered in culture from the wood.

No. 2—Untapped bark of six *Hevea* trees was lightly scraped and inoculated. After eighteen days a patch 3½" x 2" had formed and the wood was penetrated to the depth of about ½ an inch. The fungus was reisolated.

No. 3—Unwounded bark of *Hevea* was inoculated by smearing a portion of a culture over the bark and keeping it moist. In five days rubber was found on the outside of the bark, under the inoculation, and, on opening up the bark, a patch 3" x 2" was found which involved the wood to a depth of quarter of an inch. The patch was similar to those found in nature. The fungus was recovered from the wood.

No. 4—Tapped bark of *Hevea* was inoculated and Black Stripe developed in four days. The stripes were somewhat broader than those obtained with fungus No. 1.

No. 5—*Hevea* pods were flamed, put into sterile jars, wounded slightly, and inoculated by placing a little mycelium and zoospores in water near the stalk end. In seven days the pods were rotted and covered with mycelium.

No. 6—Similar to Experiment No. 5 but the pods were unwounded. In eight days the pods were beginning to rot near the point of inoculation and after ten days mycelium appeared all over the pods. The fungus was recovered in culture along with *Gloeosporium* sp. The controls remained healthy for twelve days after which they became covered with *Gloeosporium* sp. and rotted.

No. 7—Potato tubers were wounded, inoculated with mycelium, and kept in sterile jars. In six days the tubers rotted and mycelium was present in the tissues. No aerial hyphae were formed. The controls were unaffected.

No. 8—Two young papaya fruits were wounded and inoculated. There was no evident results for twelve days, after which mycelium appeared and the fruits rotted.

No. 9—Two plants of *Colocasia antiquorum* Sch. were inoculated The result was negative.

No. 10—Tomato and Tobacco plants of varying ages were wounded and inoculated, but the result was negative.

No. 11—Three young Castor Oil Plants, unwounded, were killed by the fungus in five days.

The results of the inoculations are summarised in Table 7. (+ ve = positive; — ve = negative.)

TABLE 7.

Host Plant	Wounded or Unwounded.	Point of inoculation.	Result.	Number of days.
Hevea Brasiliensis Muell. Seedlings	W	Collar and stem	+ ve	30
Hevea Brasiliensis Muell. Leaves	W	Leaf and petiole	– ve	30
Hevea Brasiliensis Muell. Bark	W	Trunk	+ ve	18
Hevea Brasiliensis Muell. Bark	U	do	+ ve	5
Hevea Brasiliensis Muell. Bark	W	Tapping cut	+ ve	4
Hevea Brasiliensis Muell. Fruit	W	Stalk end	+ ve	7
Hevea Brasiliensis Muell. Fruit	U	do	+ ve	8
Solanum Tuberosum L. ...	W	Tubers	+ ve	6
Carica papaya L. ...	W	Fruit	+ ve	12
Colocasia antiquorum Sch.	W	Leaves & collar	– ve	30
Nicotiana tabacum L.	W	Stem	– ve	30
Lycopersicum esculentum Mill.	W	do	– ve	30
Riccinus communis L.	U	do	+ ve	5

Mixed cultures and Taxonomy

No. 6 has been grown in mixed culture with Nos 1, 2, 3, 7, 8, 11, 12, 13, 14, 16, 18, 19 and 20, but oospores have not been formed. It is therefore difficult to assign No. 6 to any species. The absence of typical chlamydospores, and differences in growth on various media, separate it from Nos. 1, 2, 11, 18, 19 and 20 (*P. palmivora* Butl.) and No. 13 (*P. parasitica* Dast). In water culture and on Malt extract Agar and Potato-dextrose Agar, there is a resemblance to *P. meadii* McRae (No. 7 and No. 3.) On Green Pea Agar the aerial growth of Nos. 6, 7 and 3 is similar, and many of the spore forms are of the same type and have approximately the same ratio of length to width (Fig. 2). Nos. 6, 7 and 3 are alike in developing circular spore forms without papillae, and with thinner walls than the chlamydospores of *P. palmivora* Butl. No. 7 and No. 3 behave identically in mixed culture and are considered to be of the same species, but No. 6 has given no results in mixed culture. Morphologically, it comes nearer to Nos. 3 & 7 (*P. meadii* McRae) or No. 12 (*P. arecae* Coleman), when grown in culture in this country, than to any of the other species or strains examined, but, until oospores have been obtained, it cannot be given a specific name.

No. 8 Phytophthora sp. isolated from a disease of Hibiscus sabdariffa L. var. altissima (Roselle Fibre).

During the slump in rubber in 1922-1924 some interest was taken locally in the cultivation of Roselle fibre. A disease soon appeared, which, under certain conditions, was capable of doing considerable damage.

Symptoms.

When the plants are about two feet high or more, the stems of some of them become discolored, at soil level, by a dark brown, almost black, band, which extends slowly up the stems to a height of from three inches to more than a foot. The leaves and tips of these plants droop, but death does not take place rapidly. Sometimes the epidermis of affected plants splits, after rain, near the border of the healthy and diseased tissue, and gum exudes. The epidermis is somewhat sunken in the discoloured area on which

the cell walls form a network. The disease progresses more rapidly in the cortex than in the wood vessels, and the cortex at soil level appears to be the first part attacked. Secondary organisms e.g. *Fusarium* spp. and bacteria invade the tissues soon after they are attacked, and render isolation of the causal organism a matter of some difficulty. Some of the roots are affected near the collar, but others do not decay until the plant dies.

The tissues in the cortex of diseased plants are full of mycelium. Some of the hyphae are large and nonseptate and are mainly intercellular, and accompanying these are smaller septate hyphae. Isolations from the advancing edge of the disease usually yield a species of *Phytophthora* mixed with a species of *Fusarium*. It was found difficult to separate these two fungi, but it was eventually accomplished by inoculating rubber bark with a portion of an impure culture. The *Phytophthora* penetrated into the rubber wood and was obtained pure when reisolated.

Pure cultures of the *Fusarium* sp. failed to attack Roselle plants, and, since the *Phytophthora* sp. can reproduce the disease, it was considered to be the causal agent.

Growth in Culture.

Mycelium.—The fungus grows well on agar media, producing copious aerial mycelium on Quaker Oats Maize and Green Pea Agars. On Potato-Dextrose Agar, in Petri dish culture, the mycelium is lanate and not compact, and the growth is somewhat zoned. The hyphae vary in width from 2—10 microns, the majority being about 6 microns wide. The aerial hyphae are only slightly branched.

Sporangia.—These are formed sparingly and late on Quaker Oats and Maize Agar, but on Green Pea Agar they are present in good numbers after fourteen days. They are broadly ovate (l/w ratio = 1.25) and have prominent papillae. Zoospores are freely produced when sporangia are placed in water. In water and bean culture or in plain water large numbers of sporangia are produced in three days. They are slightly larger than those formed on Agar media and some are almost circular, but the length to width ratio is practically the same (1.23.)

Chlamydospores.—On Quaker Oats and Maize Agars brownish chlamydospores are formed in abundance after fourteen days. They are more numerous than sporangia on these media, but less numerous on Green Pea Agar. They are terminal or intercalar and range from 21-45 microns in diameter, the mean being in the neighbourhood of 30 microns on Agar media, and 36 microns in water culture.

Oospores.—No oospores have been formed in pure culture on any medium.

Measurements of sporangia and chlamydospores have been obtained from cultures on Green Pea and Quaker Oats Agar, and from water culture. These are given in Table 8. Only 30 sporangia were measured on Quaker Oats Agar as only a few had formed.

TABLE 8.

Sporangia and Chlamydospores of No. 8 Phytophthora sp. isolated from Hibiscus Sabdariffa L. from Selangor F.M.S. (1928).

SPORANGIA.

Medium.	Number measured.	Range in microns.	Mean in microns.	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
Green Pea Agar	100	30-54 x 24-45	43.57 x 35.65	1.06-1.75	1.25
Quaker Oats Agar	30	30-64 x 24-39	39.09 x 31.62	1.07-1.77	1.24
Water & Bean ..	100	30-63 x 24-51	45.05 x 36.33	1.06-1.91	1.23

CHLAMYDOSPORES.

Medium.	Number measured.	Range in microns.	Mean in microns.
Green Pea Agar ...	100	25-45	32.64
Quaker Oats Agar ...	100	21-45	30.93
Water and Bean ...	100	25.5-45	36.69

Inoculation Experiments.

No. 1—Six Roselle plants were wounded and inoculated, at soil level, with mycelium and zoospores from a culture of the *Phytophthora*. In twelve days the typical symptoms of the disease were present; the fungus had travelled seven inches up the stem. The fungus was reisolated.

No. 2—Six Roselle plants were ioculated with the *Fusarium* sp. also isolated from diseased tissues. The result was negative.

No. 3—A Roselle plant was inoculated on the stem one foot from soil level. In six days the disease was present on the stem, which had a brownish black band on four inches of the surface and in the cortex.

No. 4—Untapped Hevea bark, lightly wounded, was inoculated. Patch Canker developed in ten days, the wood being slightly penetrated.

No. 5—The tapping cuts of two rubber trees were inoculated. Black Stripe was present in four days.

No. 6—Two Hevea fruits were wounded and inoculated with the fungus. In ten days mycelium appeared on the pods which were rotted.

No. 7—Potato tubers were inoculated with the fungus. In six days the tubers rotted.

No. 8—A young papaya fruit was inoculated but remained healthy for over a month.

Mixed Cultures and Taxonomy

Since oospores have not been found in pure cultures, various mixed cultures were made and oospores were obtained in certain cases. The antheridia were always amphigynous. The results are given in Table 9. Green Pea, Maize and Quaker Oats Agar were used but Green Pea Agar was found to be most satisfactory. (+ ve = oospores present; —ve = oospores absent).

TABLE 9.

Oospores in mixed cultures of No. 8 with other species of Phytophthora.

Mixture.	Result	Number of Days.	Mean Diameter in microns.
No. 8 & No. 1 *P. Palmivora* rubber group ...	+ ve	6	22.67
No. 8 & No. 2 „ „ ...	+ ve	5	23.01
No. 8 & No. 18 „ „ ...	+ ve	6	22.69
No. 8 & No. 11 „ cacao group	– ve	30	—
No. 8 & No. 3 *P. meadii* (Malaya)	+ ve	10	23.80
No. 8 & No. 7 *P. meadii* (India) ..	+ ve	10	23.04
No. 8 & No. 12 *P. arecae* „ ...	– ve	30	—
No. 8 & No. 13 *P. parasitica* (India) ...	+ ve	4	21.16
No. 8 & No. 16 *P. nicotianae* (Sumatra) ...	+ ve	4	23.67
No. 8 & No. 6 *Phytophthora* sp. *Hevea* F. M. S. ...	– ve	30	—

From the above table it will be seen that oospores are formed when No. 8 is grown in mixed culture with members of the rubber group of *P. palmivora* Butl. (Nos. 1, 2 and 18), but not when grown with a member of the cacao group (No 11.) This indicates that the *Hibiscus* species may possibly be a member of the cacao group of *P. palmivora* Butl. Ashby,* who also obtained oospores only in the mixture with the rubber group, considers that the *Hibiscus* species is an atypical member of the cacao group of *P. palmivora* Butl. The oospores are formed in five or six days while those formed in the mixtures with No. 7 (*P. meadii*) from India and No. 3 from Malaya do not appear for ten days. Furthermore No. 8 differs from *P. meadii* in forming chlamydospores in abundance, a feature also in common with

* Private communication.

P. palmivora. Ashby has observed that some of the sporangia of No. 8 in water culture separate with a short pedicel attached in the same manner as in *P. palmivora* Butl. Consequently if Gadd's (6) theory of heterothallism in *P. palmivora* (*faberi*) is accepted, the thallus of No. 8 can be regarded as being complementary to that of a member of the rubber group of *P. palmivora* and therefore No. 8 can be assigned to the cacao group of that species. The oospores formed in mixtures of No 8 with the local and Indian strains of *P. meadii* McRae may be regarded as belonging to *P. meadii* or as hybrids in which *P. meadii*, known to be homothallic, supplies possibly the male elements and No. 8 the female elements. Gadd (11) has suggested that this is probably what occurs when large oospores appear in mixtures of small oospore forming strains of *P. parasitica* Dast and *P. palmivora* Butl.

Ashby (7) has merged *P. parasitica* Dast. and *P. nicotianae* B. de H. into one species—*P. parasitica* Dast. The writer has obtained from India *P. parasitica* Dast (No. 13), isolated from *Riccinus communis* L., and *P. nicotianae* B. de H. (No. 16), isolated from tobacco in Sumatra. These two species are identical in growth and neither of them have formed oospores in pure culture in Malaya. No. 8 is also morphologically similar to these two strains and in pure culture the three are identical in growth, and behave similarly if inoculated into *Hevea* bark (causing Patch Canker and Black Stripe), and into Roselle (causing wilt).

The similarity in the spores of the three species on Green Pea Agar is shown in Table 10.

TABLE 10.

Details of the spore forms of No. 13, No. 16 and No. 8 on Green Pea Agar.

Species.	Mean of Sporangia in microns.	Mean of Chlamydospores in microns.	Mean ratio of $\frac{L}{W}$
No. 13	45.89 x 35.97	36.19	1.26
No. 16	46.77 x 36.29	35.67	1.28
No. 8	43.57 x 35.65	32.64	1.25

The figures are practically identical in Nos. 13 and 16 and those of No. 8 are only slightly smaller.

Consequently on morphological grounds, No. 8 can be considered to be of the same species as No. 13 and No. 16 viz. *P. parasitica Dast. Sect. macrospora*. Furthermore, as shown in Table 9, oospores are formed when No. 8 is grown in mixed culture with No. 13 and with No. 16, and they are formed in four days. This suggests that No. 8 is heterothallic, and that its thallus is complementary to that of No. 13 and of No. 16 and that these are also heterothallic. But No. 13 is known to be homothallic, since it is known to form oospores in pure culture in India and in England, and No. 16 (which came from the same area in Sumatra as the species in which Ashby (7) found oospores, in pure culture, in England) may also be homothallic. Up to the present No. 8 has not formed oospores in pure culture in England, but it is possible that it may do so eventually, in which case the question would be settled, and No. 8 could not be considered to be a member of the cacao group of *P. palmivora* Butl., and the oospores formed in the mixture with that species could be looked on as hybrids. The larger oospores obtained by Ashby (5) and Lester Smith (12), in mixtures of *P. palmivora* and *P. parasitica*, were obtained from oospore forming strains of *P. parasitica* and thus were probably hybrids as Gadd suggests (11). No. 13 and No. 16 have not formed oospores in Malaya, and recently, the writer mixed them in culture with both groups of *P. palmivora*, and with each other. The result was as follows:

No. 13 & No. 1	*P. palmivora*	rubber	group	— ve	30 days.
No. 16 & No. 1	,,	,,	,,	— ve	30 ,,
No. 13 & No. 11	,,	cacao	,,	+ ve	4 ,,
No. 16 & No. 11	,,	,,	,,	+ ve	4 ,,
No. 13 & No. 16	Two strains of *P. parasitica*			— ve	30 ,,

This shews that No. 13 and No. 16 both behave similarly in forming oospores when mixed with a member of the cacao group of *P. palmivora* (No. 11). None were formed in mixtures with the rubber group, neither were any formed when No. 13 and No. 16 were mixed together. This indicates that No. 13 and No. 16 are of the same biologic strain, and that they can be regarded as atypical members of the rubber

group. of *P. palmivora* Butl. Since No. 8 can be included in the cacao group of *P. palmivora*, then the three fungi, Nos. 8, 13 and 16, can be referred to one species viz. *P. palmivora* Butl., but they can also be referred to *P. parasitica* Dast., since they are typical of the species (No. 13) which came from India as the type of the species.

The oospores formed in the mixtures of Nos. 13 and 16 with the cacao group of *P. palmivora* may be hybrids, and the result comparable to that obtained by Ashby and Lester Smith, but in that case one would expect to find oospores in the mixtures with the rubber group, but this has not happened. It would appear then, that Nos. 13 and 16, in culture Malaya, are heterothallic, and that the oospores formed are the result of a fertilisation between antheridia and oogonia from two heterothallic strains, one (No. 11) typical of *P. palmivora* Butl. cacao group and the other (No. 13 or No. 16) atypical of this species and in the rubber group. The biologic difference between Nos. 13 and 16 and No. 8 is further exemplified in mixed cultures with No. 3 and No. 7 (*P. meadii* McRae). Oospores are formed in mixtures of No. 8 with Nos. 3 and 7, but are absent when Nos. 13 and 16 are each mixed with Nos. 3 and 7.

The behaviour of *Phytophthora* spp. when grown in culture in a country different to that of origin is known to be erratic, and spore forms which are absent in one country may be present in another. What the factors are which influence this behaviour is uncertain. Oospore formation, in pure cultures of homothallic strains, is also erratic. The conclusions which can, at present, be drawn from the result of mixed cultures of the three *Phytophthorae* No. 8, No. 13 and No. 16, are:—

(1) They are members of one species, and are heterothallic in culture in Malaya.

(2) No. 8 is morphologically similar to Nos. 13 and 16, but biologically dissimilar.

(3) Nos. 13 and 16 are of the same biologic strain.

(4) Nos. 13 and 16 can be regarded as atypical members of the rubber group of *P. palmivora* Butl., and No. 8 as an atypical member of the cacao group of that species.

(5) *P. parasitica* Dast. may develop homothallic and heterothallic strains, and the latter can be regarded as being atypical members of *P. palmivora* Butl.

On acccunt of the similarity between No. 8 and Nos. 13 and 16 (*P. parasitica* Dast.), it is considered that No. 8 should be referred to this species, especially since Nos. 13 and 16 behave similarly in forming oospores, in mixed culture, with *P. palmivora* Butl. If No. 8 is considered to be atypical of *P. palmivora* Butl., then Nos. 13 and 16 should also be placed in this species; but if Nos. 13 and 16 are really strains of *P. parasitica* Dast., and therefore homothallic, this cannot be done until further information is available.

No. 9 Phytophthora colocasiae Rac. isolated from a wilt of Sireh (Piper Betle L.) at Pekan, Pahang, F.M.S.

A note on the fungus causing this disease was published in 1926 (4). Since that date the disease has not been reported from any area, and it was therefore impossible to determine whether more than one species of *Phytophthora* was present on this host in Malaya. The fungus has recently died out in culture, although attempts were made to invigorate it by inoculation on to Sireh and other hosts. The writer was enabled to study the fungus for a short period at the Imperial Bureau of Mycology, Kew, in the summer of 1926. Cultures which were grown at the Bureau grew vigorously and developed oospores freely, thus enabling the Mycologist (Mr. S. F. Ashby) to identify the fungus as *P. colocasiae* Rac. Oospores were not formed when the fungus was grown in this country, and they did not develop after the fungus was brought back from Kew and sub-cultured. On the other hand a type species of *P. colocasiae* Rac. from America, which was also brought back to Malaya, continued to produce oospores in this country.

The strain from Sireh grows well on most agar media, aerial mycelium being formed copiously. Sporangia and chlamydospores are developed fairly early and are characteristic of *P. colocasiae* Rac. Some of the sporangia are broadly ovate and others are very narrowly ovate, separating with a long slender pedicel. Zoospores are produced in the usual manner, but are formed principally by the smaller sporangia. They are large, ranging from 12—16 microns in diameter when they

round off. Active zoospores measure 12-14 x 8.5-10.5 microns. Oospores with amphigynous antheridia, are formed, in England, on Quaker Oats Agar, Maize Agar and on Bean Agar, in five days. They are coloured brownish yellow when mature, and range from 19.5—31.5 microns, those on Quaker Oats Agar being somewhat larger than the others. Chalmydospores are formed on agar media; they may be terminal or intercalar and are usually spherical with a thick wall. The diameter ranges from 18—33 microns, the mean of 100 being 24.45 microns.

TABLE 11.

Measurements of sporangia, chlamydospores and oospores of P. colocasiae Rac. from Piper Betle L. in Malaya.

SPORANGIA.

Medium	Number measured	Range in microns	Mean in microns	Range of $\frac{L}{W}$	Mean of $\frac{L}{W}$
Maize Agar ..	200	27-70 x 18-39	41.99 x 27.25	1.18-2.36	1.68
Green Pea Agar	150		40.36 x 28.43		
Quaker Oats Agar ...	100	27-72 x 18-39	43.57 x 25.89	1.27-2.40	1.70
Rubber Pods ...	50		39.62 x 24.35		
Arecanuts ..	60		39.42 x 24.35		

CHLAMYDOSPORES.

Medium	Number measured.	Range in microns.	Mean in microns.
Maize Agar ...	100	18-33	24.45

OOSPORES. *

Bean Agar ...	50	19.7-27.4	23.1
Maize Agar ...	32	19.5-26.3	22.1
Quaker Oats Agar ...	24	19.0-31.5	25.6

* Obtained from the Imperial Bureau of Mycology.

The fungus is not a vigorous parasite, except under conditions which favour it *e.g.* badly drained soil and abundant moisture. Inoculation experiments were only successful on Sireh plants when the inoculation was kept very moist. After growing in culture for some time the fungus tends to lose its virulence, and the sporangia nearly always germinate directly without forming zoospores. When first isolated the fungus was capable of causing Patch Canker of *Hevea*, if the inoculation was kept very moist for fourteen days. The patches of diseased tissue were mainly confined to the cortex. Green Areca nuts were also susceptible to attack, but, although the fungus produced sporangia on the surface of the fruit, the mycelium did not penetrate very deeply into the tissues. On rubber pods the fungus grew well and rotted the pods in six days. Plants of *Colocasia antiquorum* Sch. were inoculated, but were not attacked by the fungus. The inoculations were done, however, after the fungus had been in culture for two years, and had ceased to give positive results on Sireh and *Hevea*, so the result is not conclusive.

Control Measures.

The disease has not reappeared on the affected plots which were dug up and exposed to the sun during dry weather, and which, later, were better drained during the wet periods.

Since oospores have not been found, either in the tissues of the diseased Sireh plants or in culture in Malaya, it is probable that this treatment would be sufficient, in itself, to keep the disease in check. Should it not do so, soil disinfectants such as formalin, or Uspulun, could be applied at first, but the principal measures are adequate drainage and reduction of atmospheric moisture, by keeping down weeds and reducing shade, in wet weather.

Remarks.

Up to the present, three species of *Phytophthora* have been isolated from naturally occurring cases of Black Stripe disease of *H. brasiliensis* Muell., and inoculation experiments have shown that all three can reproduce the typical symptoms of the disease. The three species are as follows:—

(a) *P. palmivora* Butl. rubber group, (isolated frequently).

(b) *P. meadii* McRae, (isolated once).

(c) *P. heveae* Thompson, (isolated once).

These species are not all confined to Black Stripe disease; *P. palmivora* Butl. has also been obtained from naturally occurring Patch Canker, and *P. heveae* Thompson, from naturally occurring pod-rot. The species are not confined to any one locality e.g. *P. palmivora* Butl. occurs in Malacca and Perak South, and Perak North, while *P. heveae* Thompson occurs in Selangor and in Malacca.

In the case of Patch Canker of *H. brasiliensis* Muell. two species of *Phytophthora*, and one species of *Pythium*,* have been found responsible. The species are:—

(a) *P. palmivora* Butl. rubber group.

(b) No. 6 (undetermined).

(c) *Pythium* sp. (probably *P. complectens* Braun.)

In the case of Pod-rot disease of *H. brasiliensis* Muell., one species of *Phytophthora*—*P. heveae* Thompson—and the *Pythium* sp. have been found to cause the disease (1).

As far as *Phytophthora* is concerned, it cannot be concluded, however, that the species mentioned above are the only ones which cause bark and fruit disease of *H. brasiliensis* in Malaya. Inoculation experiments have shown that other species can cause the diseases mentioned, and it is highly probable that some of them will later be found naturally attacking the bark and fruit of the rubber tree.

No. 8 from *Hibiscus sabdariffa* L., No. 13 from *Riccinus communis* L. and No. 16 from Tobacco are strains of the same species and all can attack the bark of *Hevea*, giving

* The bark disease caused by this fungus has only been found in one area—Perak South. It may take the form of Collar Patch Canker. The fungus has been isolated from Patch Canker on two occasions, and from pod-rot on one occasion. It will be described in a later publication.

effects similar to the Black Stripe and Patch Canker diseases, caused by those species naturally associated with these diseases. The same result was obtained when No. 14 (from *Piper Betle* L. Pusa, India), and Nos. 18 and 19 (*P. palmivora* Butl. from coconut, Jamaica, and cotton, St. Vincent), were inoculated into Hevea. No. 11 *P. palmivora* Butl. Cacao Group) can also attack rubber bark in similar fashion. This fungus, which was isolated from coconut bud-rot in India, is a typical member of the cacao group of *P. palmivora* Butl., but no typical member of this group has, as yet, been isolated from any host in Malaya. Bud-rot of the coconut palm, caused by *Phytophthora*, has not been found in Malaya, but whether this is due to the fact that typical members of the cacao group of *P. palmivora* Butl. are not present, is a matter for conjecture. Inoculations on coconut palms with the local species of *P. palmivora* Butl. from Hevea have given negative results, while an inoculation with No. 11 was successful. These experiments require repetition as the weather was very dry for some months after the inoculations were made. No. 18 (Ashby's strain from Coconuts in Jamaica) is a member of the rubber group of *P. palmivora* Butl. This strain also failed to produce bud-rot when inoculated into coconut palms in this country, but the result is inconclusive, owing to the dry weather conditions which prevailed at the time. It is hoped to experiment further with inoculations on coconuts and other palms, when occasion permits, in order to obtain more information as to the degree of virulence of the members of the cacao and rubber group of *P. palmivora* Butl. on these hosts. Coconut plantations and rubber plantations are often located together, and in most native rubber holdings coconut palms can be found. Yet, in areas where Black Stripe disease is prevalent, adjoining coconut palms remain free from Bud-rot. Cacao is rarely grown in Malaya and *Phytophthora* pod-rot of this plant has not been encountered. *P. palmivora* (*faberi*) cacao group causes pod-rot of cacao in Ceylon, and Gadd (11) has shown that this strain will produce bud rot of wounded coconut palms almost as readily as the Ceylon coconut strain, which is also a member of the cacao group of *P. palmivora* Butl. Neither of these two strains, nor the strain from *Hevea* (rubber group), appear to be very virulent to coconuts in Ceylon, since field observations indicate that *Phytophthora* bud-rot of coconuts is not serious, even in mixed areas of coconuts, cacao and *Hevea*, when the cacao and *Hevea* trees are badly infected with canker or pod-rot.

Climatic conditions in Malaya undoubtedly influence the rate of spread and the severity of the *Phytophthora* diseases. On the western side of the peninsula, the rainfall, during the wetter months, falls mostly in the afternoons and nights, and is preceded or followed by bright sunny mornings Damp misty mornings are comparatively rare and consequently the spores of *Phytophthora*, which may have alighted on the leaves and fruit of the rubber trees, are soon dried out and killed. This is considered to be one of the main reasons why Malayan rubber plantations have remained so free from the diseases known as secondary leaf fall and pod-rot caused by *Phytophthora*, especially since No. 3 (*P. meadii* McRae), and Nos. 1 and 2 (*P. palmivora* Butl.), known to cause these diseases in India and Ceylon, have been found in Malaya. The behaviour of *P. heveae* Thompson will be watched with interest, now that it has been proved that Pod-rot of *Hevea* does occur in Malaya. Black Stripe disease which affects the tapping cut is fairly prevalent, because the overhead shade of the leaves prevents the sun from having access to the atmosphere near the trunk, and humidity remains high. The effect of this is well marked in native small holdings where overcrowding of the trees, and the presence of undergrowth, leads to increased humidity and severe attacks of bark diseases.

The disease of *Hibiscus sabdariffa* L. is rarely evident until the plants have grown sufficiently, and produced enough leaves, to give overhead shade to the soil. When this occurs the disease appears, in wet weather, in areas where the drainage is inadequate.

Black Stripe and Patch Canker diseases of the rubber tree have been dealt with by investigators in other tropical countries. In Java Rutgers (13) showed that *P palmivora* (*faberi*), obtained from Cacao and Nutmeg, could cause Black Stripe and Patch Canker of *Hevea*, but he does not mention that he isolated a *Phytophthora* from naturally occuring cases of the diseases on Hevea. Had this been attempted he would not have confused Brown Bast disease with Patch Canker, and much later confusion would have been avoided. His statement that *P. nicotianae* B. de H. would not attack the rubber tree, is interesting in view of the result obtained by the writer with No. 16 *P. nicotianae* (*parasitica*) from tobacco in Sumatra, which can attack *Hevea* bark. On the other hand, No. 17 (*P. nicotianae* (*parasitica*)), which was

isolated by Rutgers in Java and received by the writer from Kew, does not attack *Hevea* in Malaya and is probably the same species as the one used by Rutgers. This indicates that the species from Java is probably not of the same strain as the species from Sumatra. The latter can attack *Hevea* and may later be found to be a cause of Black Stripe or Patch Canker of Hevea in Sumatra. The same suggestion can be made in the case of No. 8 from Malaya and No. 13, No. 14 and No. 11 from India, since these species, on inoculation, can causes Black Stripe and Patch Canker of *Hevea* in Malaya, and may also be found to be natural responsible agents for these diseases in their respective countries. In Ceylon, *P. palmivora* (*faberi*) is recorded as the cause of Black Stripe, Patch Canker, and Pod-rot of *Hevea*, the evidence being obtained, in the case of Black Stripe and Patch Caker, as a result of inoculations with a strain of this species obtained from Cacao. The matter has been settled in the case of Pod-rot of *Hevea* by actual isolation of *P. palmivora* (*faberi*) from diseased pods (6) but there is no definite information as to whether more than one species of *Phytophthora* is normally responsible for this or the other two diseases.

Seedling Inoculations.

In the preceding pages mention is made of inoculations of *Hevea* seedlings with some of the local *Phytophthora* spp. Although the stems and collars of the seedlings were wounded before inoculation, none of the seedlings were killed. Fungus hyphae were present in the wood tissues of the inoculated seedlings and were proved to be viable, a month after the inoculation, by successful reisolations. According to Sharples et al (2), the *Phytophthora* sp. isolated from Black Stripe in 1916, was capable of killing *Hevea* seedlings, but only if they were wounded. In view of this result, it is necessary to investigate the matter further, since the possibility of attack on wounded stocks used for bud-grafting must not be overlooked.

There are no reports of a naturally occurring *Phytophthora* disease of *Hevea* seedlings from any of the rubber producing countries, and if seedlings are susceptible to attack it is strange that no such disease has occurred. The explanation is probably to be found in the fact that seedlings in nurseries are not much exposed to accidental injury, or excessive humidity, and consequently a possible *Phytophthora* attack is averted.

Summary.

1. Eight local isolations of *Phytophthora* have been studied. Of these, six were obtained from bark or fruit diseases of *Hevea brasiliensis* Muell., one was isolated from a disease of Roselle fibre plants (*Hibiscus sabdariffa L. var altissima*), and one was isolated from a disease of Sireh (*Piper Betle L.*)

2. Three species of *Phytophthora* are directly responsible for Black Stripe disease of *Hevea* in Malaya, i.e.

(a) *P. palmivora* Butl. rubber group.

(b) *P. meadii* McRae.

(c) *P. heveae* Thompson,—a new species.

Two species of *Phytophthora* and one species of *Pythium* are direct causes of Patch Canker i.e.

(a) *P. palmivora* Butl. rubber group.

(b) An unnamed species of *Phytophthora*, listed as No. 6.

(c) *Pythium* sp. (*P. complectens* Braun?).

In the case of pod-rot of *Hevea*, *P. heveae* Thompson and the *Pythium* sp. *P. complectens* Braun?) are direct causes of the disease.

3. Certain other *Phytophthorae*, from host plants other than *Hevea*, are capable of causing Black Stripe and Patch Canker of *Hevea* if artificially inoculated into the bark. These species are:

(a) No. 11 (*P. palmivora* Butl. cacao group), isolated in India, from bud-rot of the coconut palm.

(b) No. 14, (*Phytophthora* sp.) isolated, in India, from a disease of *Piper Betle* L.

(c) No. 18, (*Phytophthora palmivora* Butl. rubber group), isolated, in Jamaica, from the coconut palm.

(d) No. 19, (*P. palmivora* Butl. rubber group), isolated, in St. Vincent from a disease of cotton.

(e) No. 13, (*P. parasitica* Dast.), isolated, in India, from a disease of Castor Oil Plants.

(f) No. 16, (*P. nicotianae* B. de H. (syn *P. parasitica* Dast.)), isolated, in Sumatra, from a disease of tobacco.

(g) No. 8, (*P. parasitica* Dast.), isolated, in Malaya, from a disease of Roselle fibre plants.

4. A disease of Roselle fibre (*H. sabdariffa* L. *var altissima*) is described. The disease may be serious if drainage is inadequate. The causal organism is No. 8 (*P. parasitica* Dast.). Evidence, obtained from mixed cultures, shows that if this strain is regarded as an atypical member of the cacao group of *P. palmivora* Butl., No. 13 (*P. parasitica* Dast. India), and No. 16 (*P. nicotianae* (*parasitica*) Sumatra), when grown in culture in Malaya, may be regarded as atypical members of the rubber group of *P. palmivora* Butl.

5. The fungus which caused the disease of Sireh (*Piper Betle* L.) in Pekan, Pahang, in 1924 has been identified as *P. colocasiae* Rac. Oospores were formed when the fungus was grown in culture in England in 1926, but they have not been formed in Malaya.

6. Records have been made of the growth and morphology of the different species and strains of *Phytophthora* received, or isolated in Malaya, by the writer. These will be of value in studying any future isolations.

The writer is indebted to Professor S. F. Ashby of the Imperial Bureau of Mycology, for various cultures received, and for much useful advice in identification of the species. Thanks are also due to Dr. McRae, of the Agricultural Research Institute, Pusa, India, and to the Director of the Deli Proefstation, Medan, Sumatra, who kindly forwarded cultures.

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Note.—Since going to press a disease of Sireh (*Piper Betle* L.) has occurred in Selangor F.M.S. A *Phytophthora* has been isolated, but not yet examined in detail. It appears to be similar to the species obtained in 1924 from Sireh in Pahang i.e. *P. colocasiae* Rac. When inoculated into lightly wounded *Hevea* bark, it caused Patch Canker; the wood was deeply penetrated in five days. It also caused Black Stripe when placed on the tapping cut.

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PLATE I.

A. Sporangia and chlamydospores of No. 1 (*P. palmivora* Butl. rubber group), from Black Stripe of *Hevea*, in Malacca, S.S.

B. Sporangia and chlamydospores of No. 2 (*P. palmivora* Butl. rubber group), from Black Stripe of *Hevea*, in Perak, F.M.S.

C. Formation of oospore in mixed culture of No. 1 (*P. palmivora* Butl. rubber group) with No. 11 (*P. palmivora* Butl. cacao group).

D. *P. heveae* Thompson, showing sporangia and oospore formation.

PLATE I.

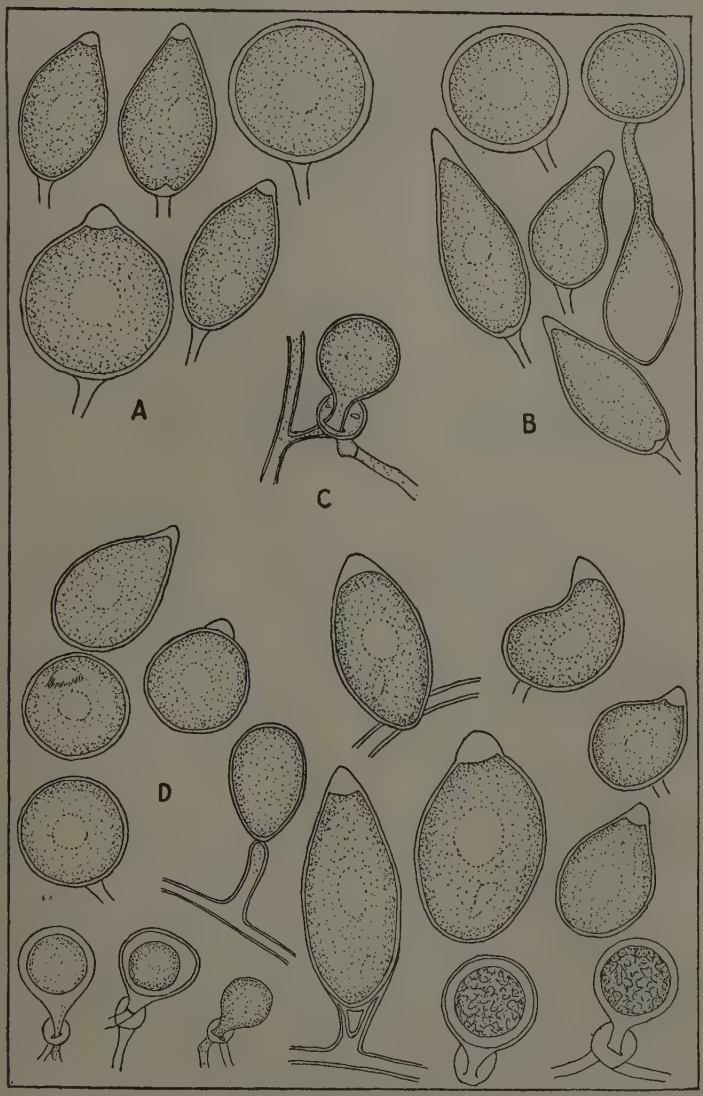

PLATE II.

D. Sporangia of No. 3 (*P. meadii*, Malaya), from Black Stripe of *Hevea* in Negri Sembilan, F.M.S.

E. Sporangia of No. 7 (*P. meadii*, India).

F. Sporangia of No. 6 (*Phytophthora* sp.), from Patch Canker of *Hevea*, in Selangor, F.M.S.

G. Sporangia of No. 12 (*P. arecae* Coleman India).

D
E
F
G

PLATE III.

Sporangia, zoospores, chlamydospores and oospores, of No. 9 (*P. colocasiae* Rac.), isolated from Sireh (*Piper Betle* L.), in Pahang, F.M.S.